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The allelopathic effects of transformed by basidiomycetes mycelia plant substrates on seeds root length

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Abstract: The influence of mycelia growth of *Trametes versicolor* 2354, *Flammulina velutipes* 2038, *Schizophyllum commune* 1769 and *Pleurotus ostreatus* 1688 on the allelopathic properties of *Corylus avellana* and *Robinia pseudoacacia* leaves was studied using seeds of *Raphanus sativus* and *Lactuca sativa* The obtained results demonstrated that investigated plant substrates changed their allelopathic properties after mycelium growth of mushrooms. The growth of *T. versicolor* 2354, on both investigated substrates resulted in inhibition of lettuce and radish root length by 15-48% as compared control. The mycelia of other studied mushroom species contributed to the intensify of the growth roots of lettuce and radish on both investigated substrates in different degrees. The strongest effect was exerted by the transformed mycelium of *F. velutipes* 2038 leaves of *C. avellana* on the growth of radish seed roots. Their length increased tenfold compared to the control. The results of this study indicate the existence of a complex of factors influencing the manifestation of allelopathic activity of biotransformed in result of mushroom mycelium growth plant substrates.

Keywords: *allelopathy, basidiomycetes mycelium, lettuce, medicinal transformed plants, radish*

INTRODUCTION

Allelopathy includes various types of interactions between different plant species, as well as between plants and microorganisms, fungi, algae and other organisms included in the ecosystem. These interactions can be positive (stimulating), negative (inhibitory) or neutral. Much attention of researchers has been paid to the allelopathy of plant extracts on various phytopathogenic fungi [Banaras et al., 2021; Kostina-Bednarz et al., 2023; Kumar et al., 2024]. The effect of dry fruiting bodies of 289 species of basidiomycetes on the germination and stem length of lettuce seeds has been established [Osivand et al., 2018]. It has been reported that most of the studied species inhibited the growth of lettuce shoots and only 26% of them stimulated their growth. It was also established that the allelopathic effect of some fungi species correlated with the presence of a number of secondary metabolites in their composition, as well as with the existence of enzymatic, antioxidant, anticancer or antimicrobial activity [Aboody Mickymary, 2020; Erida et al., 2023; Kumar et al., 2024]. We analyzed the change in the composition of biologically active compounds of some plant substrates – fruits of grapes, actinidia, sea buckthorn and mulberry leaves after mycelium growth *Ganoderma lucidum* P. Karst on them [Bisko et al., 2017]. It was found that in some transformed substrates the content of anthocyanins, the sum of chlorophylls, ascorbic acid, carotenoids or polysaccharides increases. Besides this the ratio between the content of individual biologically active substances in biotransformed sudstrates changes compared to control plant [Bisko et al., 2017].

The aim of this study was to investigate the influence of two plant substrates biotransformed as a result of mycelial growth of four species of basidiomycetes on the root length of radish and lettuce seeds.

MATERIALS AND METHODS

Mycelia of four basidiomycetes species *Trametes versicolor* 2354, *Flammulina velutipes* 2038, *Schizophyllum commune* 1769 and *Pleurotus ostreatus* 1688 from the IBK Mushroom Culture Collection of M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (NASU) [Bisko et al., 2023] (Tab. 1) were subjects. Substrates for the cultivation of the mycelia of these mushrooms were the leaves of two medicinal plants *Corylus avellana* L. and *Robinia pseudoacacia* L. from laboratory of medical botany of M.M. Hryshka National Botanical Garden, NASU. Standardized seeds of the model plants

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Table 1. List of investigated strains and species of mushrooms.

Species	Strain	Strain origin
Trametes versicolor (L.) Lloyd	2354	Isolated from a carpophore, Ukraine, Zakarpattya Region, 2014
Flammulina velutipes (Curtis) Singer	2038	Isolated from a carpophore, Ukraine, Ukraine, Melitopol, 2010
Schizophyllum commune Fr. :Fr	1769	Isolated from a carpophore, Ukraine,Kyiv, Darnitza, on *Pinus* sp., 2001
Pleurotus ostreatus (Jacq.) P. Kumm.	1688	Isolated from a carpophore, Ukraine, Kyiv, *on Quercus robur*, 2000

Raphanus sativus L. and *Lactuca sativa* L. were used in this research. Mycelia were grown on glucose peptone yeast agar (GPYA) media, g/l: glucose – 25.0; peptone – 3.0; yeast extract – 3.0; $MgSO_4 \times 7H_2O$ – 0.25; KH_2PO_4 –1.0; K_2HPO_4 – 1.0; agar – 20, distilled water – 1, pH – 6.0. The medium was sterilized using an autoclave (1 atm.). Dry leaves of *R. pseudoacacia* and *C. avellana* were crushed and were placed at 5 g in Petri dishes. Then 70 ml of distilled water was added to each Petri dish and left for 1 hour until completely absorbed. Substrates in Petri dishes were sterilized in an autoclave (1 atm.). Sterile plant substrates were inoculated with mycelia of the studied strains (inoculum diameter 1 cm). Mycelia on plant substrates grew in a thermostat at 26 ± 0.1ºC. for 14-16 days until complete overgrowth of the substrate. At the next stage, the surface of Petri dishes with substrate overgrown with mycelium was filled with a thin layer of sterile agar (20 g agar / 1 liter of water). Then 5 seeds of *C. sativa* or *R. sativus* were decomposed on the surface of these dishes at equal distances. The seeds were not subjected to additional processing before the experiment and no stratification was performed. Petri dishes with plant substrates without the addition of mycelia were used as control. Studies of allelopathic activity were carried out in a thermostat at a temperature of 26.0 ± 0.1ºC. Each experiment was conducted in fivefold. On the 3rd day, the number of germinated seeds and the length of their roots were recorded. Morphometric measurements were performed using the AxioVision 4.8.2 program. Statistical analysis was performed using Microsoft Office Excel (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

As a result of the experiments, it was found that the seeds of radish and lettuce germinated in 100% of cases in all experiment and control variants. The influence of the type of plant substrate (control variant) on the growth of radish and lettuce seed roots was demonstrated. Thus, radish roots on *R. pseudoacacia* leaves grow faster than on *C. avellane* leaves (Fig. 1, 2). The degree of stimulation or inhibition of growth of the studied seeds roots depended on some factors including the species of mushroom that grew on them. It was found that both substrates overgrown with *T. versicolor* 2354 mycelium weakly influenced the growth of radish and lettuce seeds roots in comparison with control substrates (Fig. 1, 2). The effect of two plant substrates biotransformed as a result of mycelium growth *S. commune* 1769 on the two investigated types of seeds shows the following. The allelopathic effect of *C. avellana* leaves was stronger, especially for radish seeds roots (Fig. 1, 2).

Analysis of the obtained results indicates that both studied plant substrates changed their allelopathic properties under the influence of the mycelium growth of strains of all studied species of basidiomycetes (Fig. 1, 2). At the same time, significant stimulation of the growth of roots of lettuce seeds was observed, which grew on the leaves of *R. pseudoacacia* and *C. avellana*, inoculated with the mycelium of *P. ostreatus* 1688 – their length was 4-4.4 times greater than in the control (Fig. 1, 2). The presence of the mycelium of *P. ostreatus* 1688 on both plant substrates also accelerated the growth of roots of radish seeds by 2.2-2.8 times. It was noted that the degree of stimulation of root growth of the studied seed species on substrates transformed with the mycelium of *F. velutipes* 2038 depended on the type of plant substrates (Fig. 1, 2). Therefore, the roots of radish seeds on leaves of *C. avellana*, overgrown with the mycelium of *F. velutipes* were 10 times greater, and in the case of using the leaves of *R. pseudoacacia* – 3.9 times than in the control variants (Fig. 1, 2, 3). The growth of *S. commune* 1769 mycelium also had different effects on the increase in the length of lettuce and radish roots depending on the nature of the plant substrate, but these differences

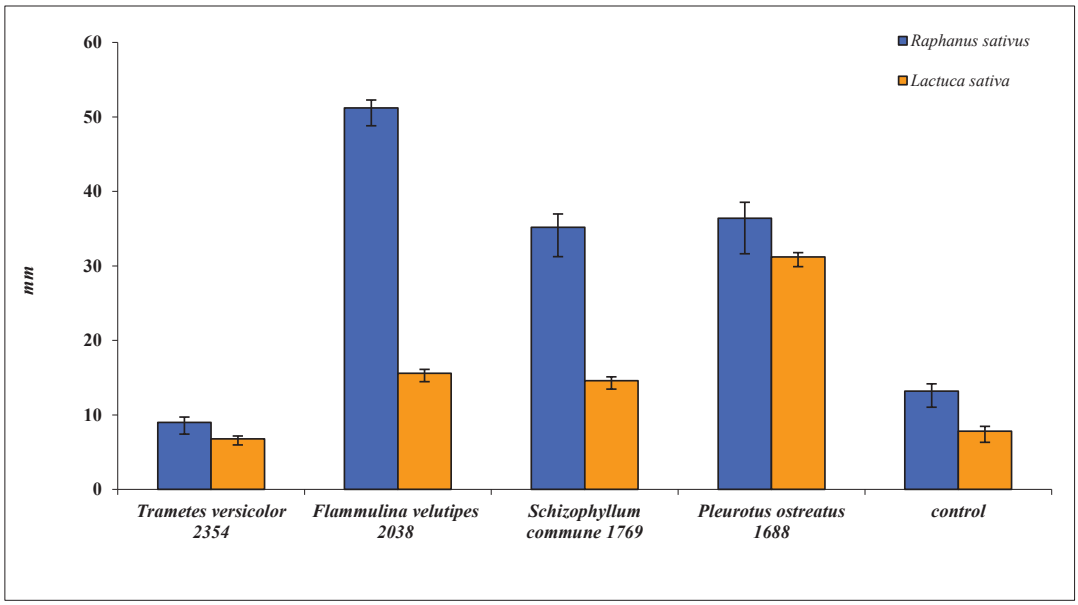

Figure 1. The influence of the leaves of transformed as a result of mycelial growth of various mushroom species on the length of *Raphanus sativus* and *Lactuca sativa* seeds roots, mm, 3rd day germination at 26.0 ± 0.1ºC.

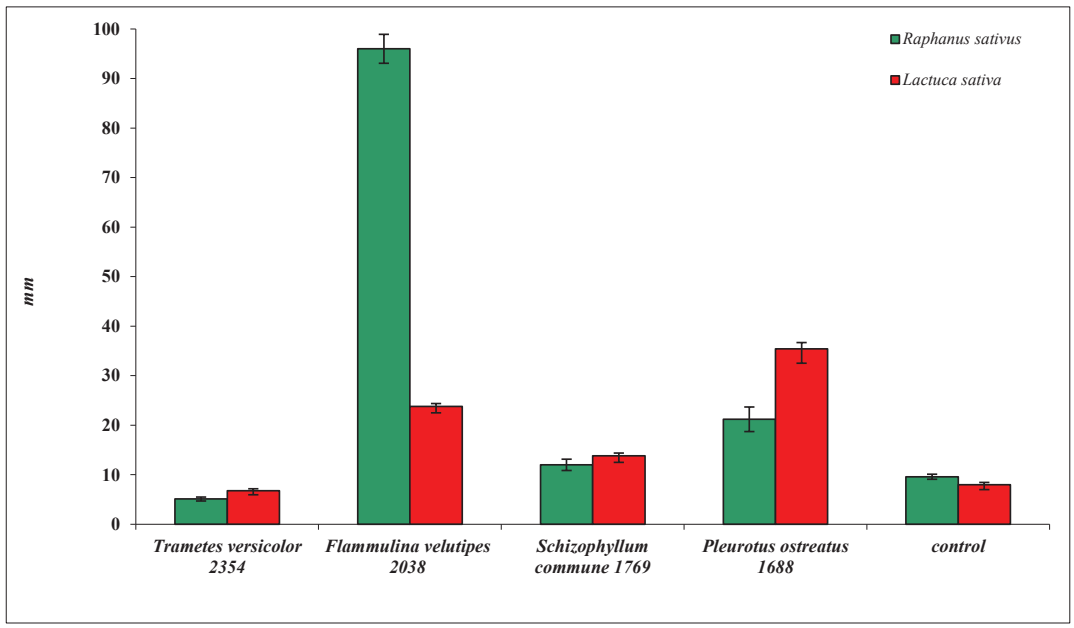

Figure 2. The influence of the leaves of *Corylus avellana* transformed as a result of mycelial growth of various mushroom species on the length of *Raphanus sativus* and *Lactuca sativa* seeds roots, mm, 3rd day germination at 26.0 ± 0.1ºC.

were not as significant as in the case of *F. velutipes* 2038 mycelium (Fig. 1, 2).

Data were previously obtained to testify about the allelopathic effect of dry biomass of 9 strains 6 species *Ganoderma* P. Karst. on the root growth of cucumber and lettuce seeds. Depending on strain biological properties it was different – from suppression by 82.4% to stimulation by 49% compared to the control [Boromensky et al., 2021a, 2021b]. The same authors showed a similar inhibitory effect of aqueous, ethyl acetate and methanol extracts of dry biomass of two strains two species *Ganoderma* on the root growth of

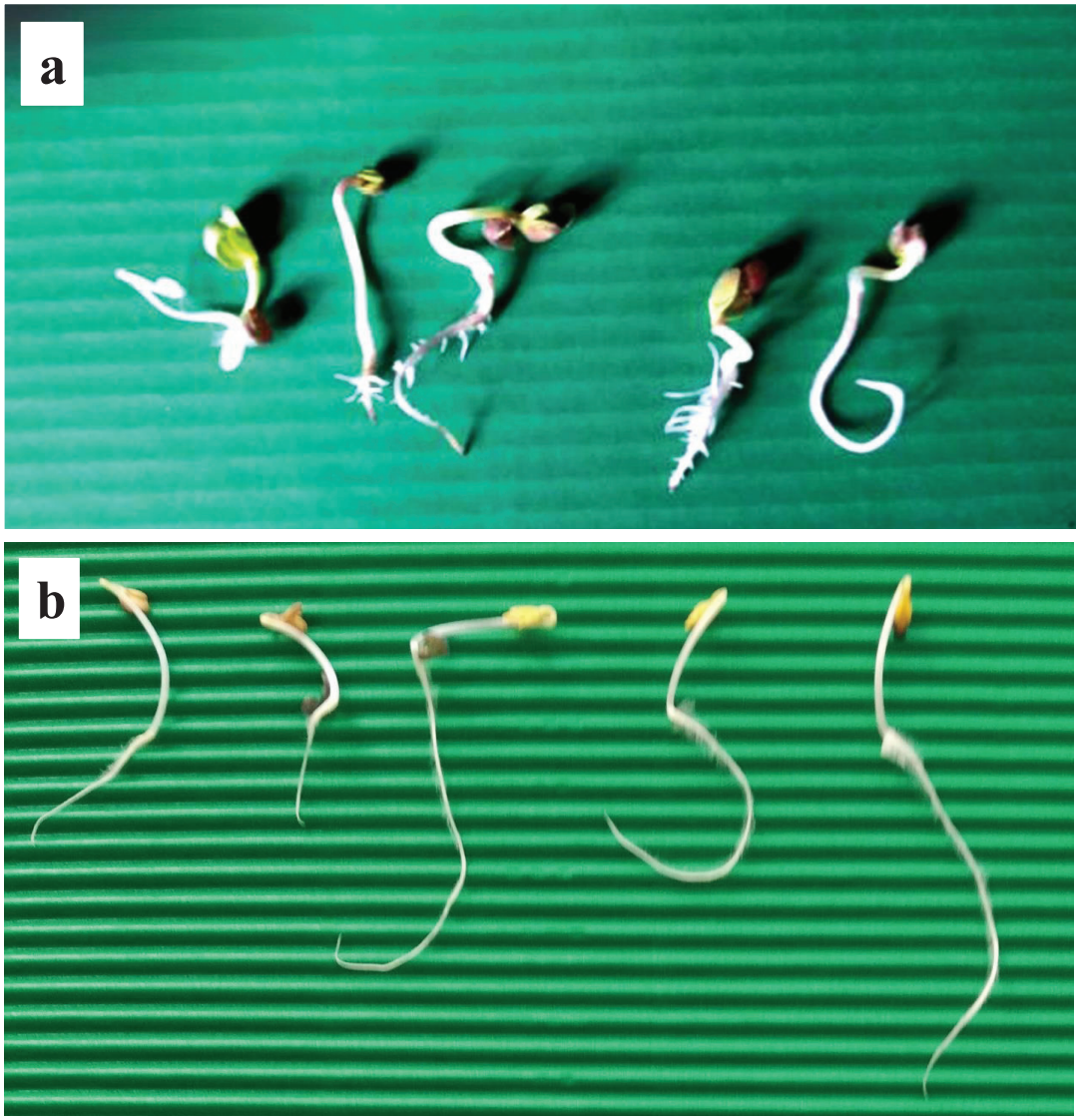

Figure 3. The influence of the leaves of *Corylus avellana* on the length of *Raphanus sativus* seeds roots on 3rd day germination at 26.0 ± 0.1ºC, mm: a – control leaves, b – leaves after growth of *Flammulina velutipes* 2038 mycelium.

lettuce seeds [Boromenskyi et al., 2021b]. The results of the allelopathic effect of seven strains of the species *Pholiota* (Fr.) P. Kumm dry biomass on the root growth of cucumber and lettuce seeds demonstrated that, depending on the biological characteristics of the strain, the inhibition effect can be 8.6 – 91.8% compared to control seeds [Regeda et al., 2025].

Thus, our results agree the conclusions made by previous researchers about the decisive role of species and strain biological characteristics of basidiomycetes associated with the allelopathic activity on vegetable seeds. We received date for the first time about the allelopathic activity changes of plant substrates biotransformed by basidiomycete mycelium on the growth of vegetable seeds roots.

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Toxum köklərinin uzunluğuna bazidiomiset miselisi ilə transformasiya olunmuş bitki substratlarının allelopatik təsiri

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Trametes versicolor 2354, *Flammulina velutipes* 2038, *Schizophyllum commune* 1769 və *Pleurotus ostreatus* 1688-in ştammlarının miselial inkişafının *Corylus avellana* və *Robinia pseudoacacia* yarpaqlarını istifadə etməklə allelopatik təsiri *Raphanus sativus* L. və *Lactuca sativa* toxumlarında araşdırılmışdır. Alınmış nəticələr göbələklərin miselisi inkişaf etdikdən sonra tədqiq edilən bitki substratlarının allelopatik xassələrinin dəyişdiyini göstərmişdir. Hər iki tədqiq edilmiş substratda *T. versicolor* 2354 ştammının inkişafı nəzarətlə müqayisədə kahı və turp kökünün uzunluğunun 15-48% inhibə edilməsi ilə nəticələnmişdir. Digər tədqiq edilmiş göbələk növlərinin miselisi hər iki istifadə edilmiş substratda kahı və turpun köklərinin böyüməsini müxtəlif dərəcədə intensivləşməsinə səbəb olmuşdur. Turp toxumunun köklərinin böyüməsinə ən güclü təsiri *C. avellana* yarpaqlarının *F. velutipes* 2038 ştammı ilə transformasiya olunmuş miselisi göstərmişdir. Onların uzunluğu kontrolla müqayisədə 10 dəfə artmışdır. Bu tədqiqatın nəticələri göbələk miselisinin bitki substratlarının böyüməsi nəticəsində biotransformasiya olunmuş allelopatik aktivliyin təzahürünə təsir edən amillər kompleksinin mövcudluğunu göstərir.
Açar sözlər: *allelopatiya, bazidiomiset miselisi, kahı, transformasiya olunmuş dərman bitkisi, turp*

Аллелопатическое влияние рансформированных мицелием базидиомицетов растительных субстратов на длину корней семян

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Изучали влияние роста мицелия *Trametes versicolor* 2354, *Flammulina velutipes* 2038, *Schizophyllum commune* 1769 и *Pleurotus ostreatus* 1688 на аллелопатические свойства листьев *Corylus avellana* и *Robinia pseudoacacia*, используя семена *Raphanus sativus* и *Lactuca sativa*. Полученные данные показали, что рост *T. versicolor* 2354 на обоих исследуемых субстратах привел к ингибированию длины корней салата и редиса на 15-48% по сравнению с контролем. Мицелий других исследуемых видов грибов способствовал интенсификации роста корней семян салата и редиса на обоих изученных субстратах в разной степени. Наиболее сильное влияние на рост корней редиса оказали трансформированные мицелием *F. velutipes* 2038 листья *C. avellana* - их длина увеличилась в 10 раз по сравнению с контролем. Наши результаты подтвердили данные других исследователей о наличии комплекса факторов, влияющих на проявление аллелопатической активности растительных субстратов. Нами впервые установлено значительные изменения аллелопатической активности растительных субстратов в результате роста на них мицелия базидиальных грибов.

Ключевые слова: *аллелопатия, мицелий базидиомицетов, цикорий, трансформированное лекарственное растения, редис*